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**Phytochemical Screening of the Ethanolic Extracts of Seeds
of *Momordica charantia* L.**

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Abstract

Momordica charantia, commonly used in Indian sub-continent known for its medicinal properties. It belongs to the family of cucurbitaceae and widely used against many diseases since ancient times. The aim of this study was to extract the phytochemical compounds of bitter melon. The herb is also a rich source of various phytochemicals like bioactive alkaloids and glycosides etc; still its activity over Cancer and HIV is unknown. Extraction of polyphenolic compounds was carried out using phytochemical screening which shows presence of different compounds such as flavonoids, saponins, carbohydrates, tannins, and terpenoids. On the basis of all the qualitative tests performed in each extracts; ethanolic extract was subjected for the further phytochemical estimation.

Key-Words: *Momordica charantia*, Ethanolic Extract, Phytochemicals

Introduction

Bitter melon, *Momordica charantia* L. is a member of the Cucurbitaceae family. The latin name "Momordica" means 'to bite' and refers to the leaves of the bitter melon plants, which have jagged edges and look like they have been bitten [1]. It is also referred to by different names around the world: balsam pear (English), Karella (Hindi or Urdu), Nigauri or Goya (Japanese), Ku gua (Madarin), Ko guai (Taiwanese), Kho qua (Vietnamese), Ampalaya (Philippines) and Assorossie (French). Generally, the bitter melon fruit has an oblong cucumber-like shape, ranging from 9 to 60 cm long, but in contrast to cucumbers, it has a very warty-looking exterior [2]. Bitter melon is widely cultivated in tropical and subtropical countries, where it is a popular traditional medicinal fruit. In the scientific literature, it has been linked with a wide range of therapeutic effects, including anticancer [3], anti-viral [4], anti-inflammatory [5], hypolipidaemic [6], hypocholesterolaemic [6], immuno-modulatory [7] and anti-diabetic [8] properties. Studies have reported that different varieties of bitter melon may differ in their content of bioactive compounds [9,10].

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However, some of the proposed therapeutic effects have been in part attributed to its content of flavonoids [7,8,11]. Flavonoids occur naturally in plants either linked to sugars (glycosides) or without the sugars (aglycones). Flavonoid aglycones are usually extracted with less polar solvents, such as benzene, chloroform and diethyl ether [13] while flavonoid glycosides are commonly extracted with more polar solvents, such as acetone, butanol, methanol and ethanol [14-16]. Ethanol [11,17], water [11] and methanol [18] have been used for extracting flavonoids and other bioactive compounds from bitter melon. However, studies on the use of different solvents, including water and less polar organic solvents, for the extraction of flavonoids from different bitter melon varieties are still limited. For example, the ratio of flavonoid aglycones to glycosides may differ between varieties and thereby lead to different extraction efficiencies, depending on the type of solvent used [13-16]. Herbal products seem to be an unexplored domain whereby a large number of pharmaco-therapeutic agents can be isolated and screened to determine therapeutic activity. *Bitter melon* is one of the widely used herbs, used as condiment in India; the plant is reported to have antidiabetic, analgesic, anticancer potential. The drug is used as insulin deficiency aphrodisiac, tonic, diuretic, expectorant, Diabetes, and useful in inflammation, Ulcer, cancer pains, Microbes, sour eructation's, tuberculosis, diseases of kidney catarrhal affections to destroy bad smell in mouth and other part of the body. Therefore the aim of the current work is to determine active phytochemical present in the ethanolic extract.

Material and Methods

Plant Materials

The fresh whole plant of *bitter melon* was purchased from local market. It was then identified and authenticated by Dr. A.B. Tiwari, Sr. Scientist, Department of Crop and Herbal Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P).

Extraction

The plant was shade dried at room temperature and then ground into fine powder. The powder was sieved to have a uniform size. 25 gm of dried powder was extracted with different solvents viz, pet. ether, benzene, chloroform, acetone, ethanol and finally with water in soxhlet apparatus. (Figure 1). The extract was filtered using a whatman filter paper no. 4 and concentrated at 40°C; dried extract was refrigerated at 4 °C until use. The extract was concentrated and dried to a constant weight. The dried extracts of the drugs were evaluated for physical parameters such as consistency, color, odor and taste. The presence of desired phytochemicals i.e. flavonoids, tannin. Qualitative chemical tests were carried out for the ethanolic extract of *Bitter melon*. The results of the tests showed the presence of carbohydrates, reducing sugars, saponins, phenolics, tannins, and flavonoids.

Chemicals

Methanol and acetone were obtained from Merck Company. Ethanol was purchased from Fronine and the n-Butanol Sodium nitrite, aluminium chloride, sodium hydroxide and rutin were purchased from Sigma-Aldrich Company pvt.ltd

Preliminary phytochemical investigations

The preliminary phytochemical investigations were carried out with ethanolic extracts of seeds of bitter melon for qualitative identification of phytochemical constituents present with each extract and tests were carried out by following standard methods. All the chemicals and reagents used were of analytical grade. On the basis of all the qualitative tests performed in each extracts; ethanolic extract was subjected for the further phytochemical and pharmacological studies because only the ethanolic extracts and Saponins

Total Flavonoid Content

The total flavonoid content (TFC) of the bitter melon extracts was determined as described by Wu and Ng [11]. Briefly, 0.5 mL of diluted sample was mixed with 2 mL of deionised water followed by the addition of 150 µL of 5% (w/v) sodium nitrite solution. After 6 min, 150 µL of 10% (w/v) aluminum chloride was added and the mixture was incubated for another 6 min. Then, 2 mL of 4% (w/v) sodium hydroxide was added and the solution was immediately made up to 5

mL with deionised water, mixed thoroughly and placed in the dark at room temperature

for 15 min. The absorption of the solution was measured at 510 nm against a reagent blank. Rutin was used as a standard and the TFC was expressed as mg of Rutin Equivalents (RE) per g of bitter melon powder on a dry from 5°C to 30°C, where it reached a plateau, and then decreased at temperatures above 50°C, the latter possibly due to degradation of the flavonoids [12]. Nevertheless, significantly more flavonoids were extracted at the high temperatures (60°C - 90°C) compared to the low temperatures (5°C - 20°C) (Figure 2). It is well known that heat increases the solubility and diffusion coefficients of solutes, like the flavonoids, and decreases the viscosity of the extracting solvent, especially water. High temperatures can also result in the cell wall of plant materials, such as bitter melon, becoming more permeable to solvent and therefore, the inner cell components can diffuse more easily into the solvent [19,21]. Since the TFC reached a plateau (Figure 3) between 30°C and 50°C (0.8 - 0.9 mg RE/g), the optimal temperature was chosen to be 40°C (0.87 mg RE/g) and used for the subsequent optimization experiments.

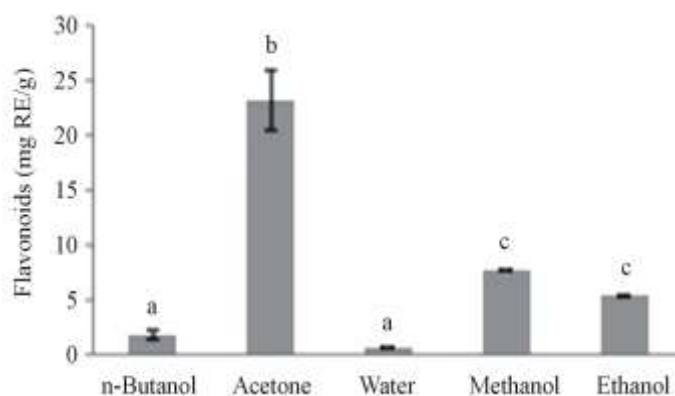


Figure 1. The extraction of flavonoids with five solvents. bitter melon powder (1 g) was extracted with 100 mL of each solvent for 1 h at 80°C for ethanol, n-butanol and water and at 50°C and 60°C for acetone and methanol, respectively. Values are means \pm SD of flavonoids extracted per gram of bitter melon powder and those not sharing a letter are significantly different ($P < 0.05$).

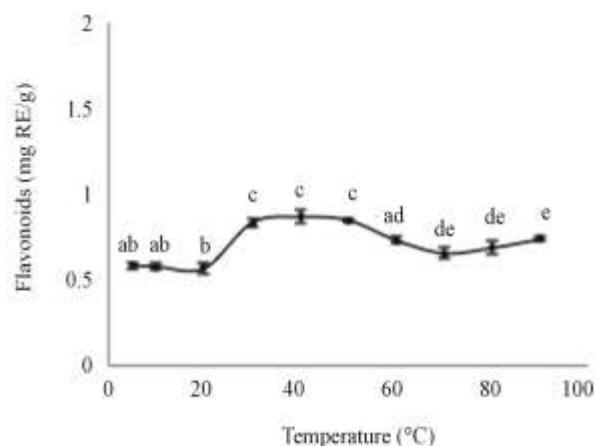


Figure 2. Effect of temperature on the aqueous extraction of flavonoids. Moonlight bitter melon powder (1 g) was extracted with 100 mL of water for 1 h at 80°C. Values are means \pm SD of flavonoids extracted per gram of bitter melon powder and those not sharing a letter are significantly different ($P < 0.05$).

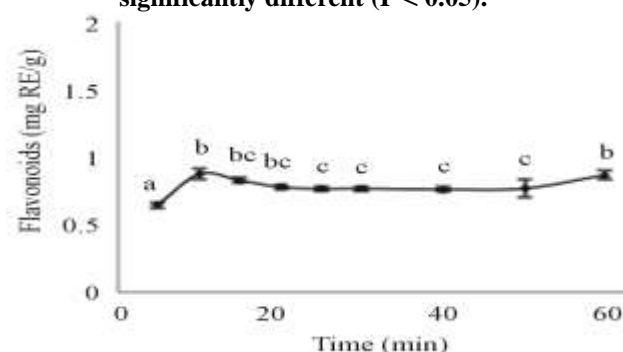


Figure 3. Effect of time on the aqueous extraction of flavonoids bitter melon powder (1 g) was extracted with 100 mL of water for 1 h at 40°C. Values are means \pm SD of flavonoids extracted per gram of bitter melon powder and those not sharing a letter are significantly different ($P < 0.05$).

Results and Discussion

The products of natural origin i.e the immense treasure provided by the nature as plants are a source for bioactive compounds and have potential for developing some novel therapeutic agents. Over the last decade there has been a growing interest in drugs of plant origin and such drugs formed an important class for disease control. Among the known plant species, only a small percentage has been investigated for their phytochemicals and pharmacological activities. Adverse side effects and high cost of modern medicine has intensified the search for other effective alternatives. Natural products in general and medicinal plants in particular, are believed to be an important

source of new chemical substances with potential therapeutic efficacy. *Bitter melon* were subjected to extraction and phytochemical screening (Table I & Table II) to ascertain the results of phytochemical screening. The phytochemical screening shows the presence of alkaloids, flavonoids and tannins. Herbal drugs are derived from heterogeneous sources leading to variations. This makes the standardization of herbal medicines all the more important as erroneous results can cause variations in pharmacological and phytochemical studies.

Conclusion

Today we are witnessing a great deal of public interest in the use of herbal remedies. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. This work was conducted to explore the hidden potential of this unexplored herb. Initially due to the less data available in this plant we felt tough to decide the basis of our work, so the strong basis was opted to work on that plant was the other species of the same genera and phytochemical reported on this plant mainly flavonoids. In recent findings done on these plants as well as flavonoids It was thought worthwhile to investigate and to provide the scientific data on its use as an antiulcer agent. The work was initialized by the collection of its seeds, which was not a daunting task. The successive solvent extraction was the primary work done in it, the extractive value of all the extracts except the ethyl acetate and ethanolic extract were in workable quantities. Qualitative tests revealed the fact that ethanolic extract of this plant contains major phytochemicals viz. phenolics, flavonoids, tannins, saponins, and traces of alkaloids. It was thought worthy to select this extract for the further studies.

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Table I: Extractive values of Bitter melon

S.No.	Name of Extract	Colour	Values in Percentage
1.	Petroleum ether	Dark Brown	3.52
2.	Benzene	Light Yellow	2.80
3.	Chloroform	Light Brown	0.30
4.	Acetone	Reddish Brown	0.23
5.	Ethanol	Dark Red	3.24
6.	Water	Brown	12.31

Table II: DETAILS OF QUALITATIVE PHYTOCHEMICAL TESTS

S.No.	Tests	PE	B	C	A	E	W
1.	Tests for Steroids						
	Test solution+Conc H ₂ SO ₄	+	-	-	-	+	-
	Salkowski’s test	-	+	-	-	+	+
2.	Test for Glycosides						
	Keller–killiani test	-	+	+	-	+	-
	Fehling’s reagent	+	-	-	+	+	-
3.	Tests for Saponins						
	Frothing Test	+	-	-	-	+	-
	Haemolysis test	+	-	-	-	-	-
4.	Test for Carbohydrates						
	Molish’s Test	+	-	-	+	+	+
	Barfoed’s Test	-	-	+	-	+	-

5.	Tests for Alkaloids						
	Mayer's Test.	-	-	-	-	+	+
	Dragendorff's Test	-	-	-	-	+	
6.	Tests for Flavonoids						
	Ammonium Test	-	-	-	+	+	-
	Zn-HCl reduction Test	-	-	-	-	+	+
	Lead Acetate Test	-	-	-	+	+	+
7.	Tests for Tannins						
	Lead Acetate Test	-	-	-	+	+	-
	Di HNO ₃	-	-	-	+	-	-
8.	Tests for Amino acids and Proteins						
	Million's Test	-	-	-	-	+	-
	Ninhydrin Test	-	-	-	-	-	-
9.	Tests for Fixed oil and Fats						
	Spot test	+	-	-	-	-	-

+ =Present; - = Absent

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